

	PCR 20 µL reac	Final Concentration			
5X Phusion HF Buffer	4	1X			
10 mM dNTPs	0.4	200 µM			
10 µM Forward Primer	1	0.5 µM			
10 µM Reverse Primer	1	0.5 µM			
G-block (0.1ng/µL)	1				
DMSO (optional)	0.6	3 %			
Phusion DNA Polymerase	0.2	1.0 units/50 µl PCR			
Nuclease-free Water	11.8				
Total volume	20				
Number of reactions (including controls):					
WORK ON ICE					
number of reactions:	2				
Mastermix without primers	volume in uL				
5X Phusion HF Buffer	8		negative ctrl (water)	CYC A-	
10 mM dNTPs	0.8		CYC_3 and c3_3	8 A	
DMSO (optional)	1.2		amplicon is CYC		
Phusion DNA Polymerase (ADDED LAST)	0.4		forward primer	f_GBS_CYC	
Nuclease-free Water	23.6		rev primer	r_GBS_CYC	
Volume of master mix in each tube:	17 uL				
In each tube, add :					
10 µM Forward primer	1uL				
10 µM Reverse primer	1uL				
G-block (0.1ng/µL) (or water for control)	1				
Sample A					
PCR programming "YEAST-A":					
Initial Denaturation	98°C	30 seconds			
	98°C	10 seconds			
	58°C	30 seconds			
35 Cycles	72°C	30 seconds			
Final Extension	72°C	10 minutes			
Hold	4°C				